ACTA UNIVERSITATIS UPSALIENSIS

Abstracts of Uppsala Dissertations in Medicine

14

LYMPHOID CELLS IN ALLERGIC CONTACT DERMATITIS IN GUINEA-PIGS

By

OVE GROTH

UPPSALA 1964

LYMPHOID CELLS IN ALLERGIC CONTACT DERMATITIS IN GUINEA-PIGS

AKADEMISK AVHANDLING

som med vederbörligt tillstånd av Medicinska Fakulteten
vid Uppsala Universitet för vinnande av medicine doktorsgrad
framlägges till offentlig granskning å
hudklinikens föreläsningssal
lördagen den 25 april 1964 kl. 9 f.m.

AV

OVE GROTH
Med. lic., Norrl.

UPPSALA 1964
ALMQVIST & WIKSELLS BOKTRYCKERI AB

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41550018_0046

From the Departments of Dermatology (Head: Professor N. Thyresson) and Histology (Head: Professor K.-E. Fichtelius), University of Uppsala, Sweden

Lymphoid Cells in Allergic Contact Dermatitis in Guinea-pigs

by

OVE GROTH

This review is based on the following papers,

I. GROTH, O.: The epidermal infiltration of lymphoid cells in allergic contact dermatitis. A quantitative approach in guinea-pigs. *Acta Dermatovener. 44,* 1, 1964.

II. GROTH, O.: The selection of lymphoid cells in allergic contact dermatitis in guinea-pigs. *Acta Dermatovener. 44,* 21, 1964.

GROTH, O.: Studies on contact skin reactions and normal skin of passively sensitized guinea-pigs.

III:1. *Part I.* A histological study of test lesions in recipients after passive transfer of dinitrochlorobenzene (DNCB)-hypersensitivity with lymphoid cells.

III:2. *Part II.* A microautoradiographic study of test lesions and normal skin after passive transfer of dinitrochlorobenzene hypersensitivity with H^3-labelled lymphoid cells. *Acta Soc. Med. Upsal. 68,* 193, 1963.

Introduction

Since the pioneer work of Frei (*27*), Bloch & Steiner-Wourlich (3), and others, animal experiments, chiefly with guinea-pigs, have been carried out to elucidate problems of contact allergy (for ref. see 62).

Contact allergy is commonly induced by the application of simple chemicals to the skin, or by injecting them into that organ. The sensitizing properties of these substances seem to be essentially dependent on their protein-binding capacity (17, 46, 49, see also 11, 16).

R. UNIV. BIBLIOTHEEK LEIDEN

In addition to inducing contact allergy, however, they also give rise to the production of antibodies demonstrable in the serum (9, 10, 46, see also 62). The route and manner of administration of the antigen seem greatly to determine the type of sensitization, immediate or delayed, that will predominate. Experimental investigations suggest that different cells may be responsible for the two reactivity states (57).

Experience in the clinical field too indicates that delayed hypersensitivity may arise in cells other than those engaged in the synthesis of humoral antibodies. For example, patients with hypogammaglobulinaemia with no demonstrable antibodies in the serum have developed contact allergy that has proved passively transferable with cells to normal individuals (32). The opposite, with normal antibody response in serum but inability to elaborate a delayed response, has been reported in sarcoidosis (19) and in Hodgkin's disease (18, 23, 58).

The most important cells concerned in the synthesis of the antibodies involved in immediate hypersensitive reactivity are "transitional cells" (22) or plasmocytes. It is not clear what cells respond with delayed reactivity, but there is much to suggest that they belong to the lymphoid-cell series (57).

Interest in the part played by the lymphoid cells in delayed hypersensitivity appears to have been roused when Landsteiner & Chase (45) and Chase (8) succeeded in passively transferring to normal guinea-pigs contact and tuberculin allergy with the aid of cell suspensions prepared from sensitized guinea-pigs. Haxthausen postulated on the basis of his own investigations (39, 40) that the passive transfer factor of contact allergy was probably to be found in the lymphocytes. This must be regarded as having been confirmed by the passive-transfer experiments carried out by Wesslén (70) and Skog (61), who used thoracic-duct lymphocytes in tuberculin and contact allergy respectively.

The presence of lymphoid cells would also seem to be necessary in order to be able to provoke a contact-allergic reaction in a sensitized guinea-pig. This has been shown by provoking lymphopenia by means of X-rays (6, 14) or with lymphocyte immune serum (42, 67, 69). The intensity of a contact-allergic test reaction under such circumstances is reduced or eliminated parallel to the fall in the number of circulating lymphocytes (in contrast to the toxic reaction, which remains unaffected). Maguire and Maibach (48), however, were unable to confirm the finding that X-ray-induced lymphopenia specifically attenuates the allergic contact dermatitis reaction.

The reaction area in different forms of delayed sensitivity is characterized by a largely mononuclear-cell infiltration. These cells that during the early stages are localized about the vessels may well be derived from haematogenous lymphocytes (20, 29, 31, 65). In the epicutaneously induced contact-allergic reaction this cellular infiltration starts in

the corium, subsequently to become localized to the part bounding on the epidermis, and in the guinea-pig reaches its maximum after 24–48 hours. Since delayed sensitivity to simple chemicals has so far not been demonstrated in tissues other than the skin and adjacent mucous membranes, investigations have been done to elucidate whether cells already present in the skin (and chiefly epidermal cells) may be primarily engaged and may give rise to reactions on the administration of antigen. Available evidence suggests that this is not the case (20, 38).

Among the very great number of morphological investigations that have been carried out, opinions have differed concerning in which cells the first changes appear. The various and to some extent contradictory views that have been published are discussed at length by Bandmann (2), G. Miescher (51), and others. Waksman has discussed in detail the cytotoxic effect commonly seen in antigen-antibody reactions (64). On the basis of morphological and other investigations Waksman concludes, "There is now strong evidence that the primary event in hypersensitive reactions of the 'delayed' type is a reaction of 'sensitized' mononuclear cells, probably coming from the circulation, with antigen. This reaction appears to consist of local accumulation and proliferation of these cells. When tissue damage occurs, it is clearly secondary to the mononuclear-cell reaction" (66). Like Baer *et al.* (1), Fischer and Cooke (26), and others, he believes that in allergic contact reactions the round-cell infiltration (lymphocytes and histiocytes) is primary and that the epidermal changes are secondary to the antigen–mononuclear-cell reaction (65).

When in delayed sensitivity a parallel to the humoral antibodies is sought among the lymphoid cells (cellular antibodies) it must be stressed that there is still no conclusive evidence of the existence of cell-bound, cell-fixed, or any other type of cellular antibodies (31).

In the light of the above it would seem important further to elucidate the behavior of the lymphoid cell in the delayed allergic skin reaction. In the investigations now presented the study of the lymphoid cells has been limited to the region of the contact-allergic reaction, the reaction having been provoked by epicutaneous testing in guinea-pigs actively and passively sensitized to DNCB.

The intension was to label lymphoid cells of contact-sensitized animals, using tritiated thymidine, and with the aid of autoradiographic technique to study and possibly also to count the labelled lymphoid cells in the test lesions under different experimental conditions. Similarly it was planned to study the test lesions in animals passively sensitized by transfusion of isotope-labelled lymphoid cells from sensitized donors.

Autoradiography by the stripping-film technique did not allow of systematic counting of cells in the corium, however. Further, it proved

difficult to distinguish between different cells in this tissue. These problems were less prominent in the epidermis, however. The relationship between lymphoid cells and polymorphonuclear cells in the test lesions has been examined by a number of researchers (for ref. see 62), but no form of quantitative assessment of the lymphoid-cell infiltration in the reaction areas would seem to have been carried out either in the corium or epidermis. Numerical elucidation of the lymphoid-cell infiltration (Paper I), especially with regard to the epidermis, is therefore important, and indeed essential, in order to be able to assess the results of the microautoradiographic investigations (Papers II and III: 2).

Paper I

This investigation was primarily aimed at obtaining a measure of the lymphoid-cell infiltration in the allergic contact reaction in skin, to form a basis for grading the intensity of the reaction.

Since the predominant cellular infiltration in the fully-developed, 24–48-hour-old test lesion in guinea-pigs is localized to the upper corium, it would seem most logical to make a numerical assessment of the cells in that tissue. The counts that were attempted proved very time-consuming, however, even over small areas. The results were unreliable, partly owing to the difficulty in classifying the cells. Representative figures for the lymphoid-cell infiltration in test lesions could therefore not be reached by means of counts made in the corium. A rough appreciation of the cellular infiltration in the corium was carried out by means of general microscopy, however.

In high-power magnification of sections of skin showing allergic contact dermatitis it can be seen that the epidermis too is infiltrated by lymphoid cells, and that these are uniform and characteristic in appearance and are usually readily distinguishable from epithelial cells. A method was therefore worked out to obtain a measure of the lymphoid-cell infiltration in the epidermis. The cells were counted under a magnification of × 1000. All cells in the epidermis, apart from cells at the openings of the hair follicles, were classified and counted. One-thousand cells were counted in each skin sample, one or sometimes two sections being used to reach this number. The degree of lymphoid-cell infiltration is given per thousand of all cells in the epidermis. These figures are directly comparable for normal and tested skin in the same animal. Since the number of lymphoid cells in normal skin varies somewhat among individual animals, the increase in lymphoid cells in the test areas in each individual animal was calculated by reducing the gross lymphoid-cell count per thousand by the number

of lymphoid cells per thousand in the normal skin of the same animal. The resultant figure is the *lymphoid-cell response in epidermis,* and is used as a measure of the intensity of the reaction.

The differential epidermal cell count used here places great demands on the quality of the sections. This has in turn necessitated the development of a careful, reproducible histological technique. The greatest difficulty has been to find a method of preparation in which shrinkage of cells and tissue is kept to a minimum, but which gives thin, entire sections.

In the assessment of allergic contact reactions by means of the lymphoid-cell response the error of the method has been largely due to unevenness and variation in the lymphoid-cell infiltration within the test lesion.

The skin reaction was also assessed by naked-eye inspection, and was graded in the standard fashion with regard to redness and induration (0, +, + +, + + +).

An attempt at grading the reaction in accordance with the general microscopical appearance was also made. Regard was chiefly paid to the intensity of the cellular infiltration in the corium (see above), but account was also taken of the presence of acanthosis and spongiosis in the epidermis.

Albino guinea-pigs were sensitized by epicutaneous application of 2,4-dinitrochlorobenzene (DNCB) on two successive days. The animals were tested after 11 (group B), 15 (group C), and 25 days (group D). In two test areas per animal 9 and 20 γ DNCB dissolved in acetone was applied per sq. cm (29). Among these animals most of the tests were assessed as positive by naked-eye inspection and general microscopy, and there was as a rule a moderate or marked lymphoid-cell response. Variation in the intensity of the reaction between different animals was noted with all three methods, but the lymphoid-cell response offered the best means of grading the results. An advantage of this last-named method has been apparent on comparison of the results of assessment of tests with 9 and 20 γ DNCB, where the lymphoid-cell response showed a more definite increase in relation to the dose of antigen.

On comparison of the different methods of assessment in individual reactions the difference appears chiefly in the grading of the intensity of the reactions. There seems to be a closer aggreement between the findings on general microscopy and the lymphoid-cell response than between the naked-eye findings and the lymphoid-cell response. This suggests that the lymphoid-cell infiltration in the corium is fairly accurately reflected in the lymphoid-cell infiltration in the epidermis.

A comparison was made of the test results in the three groups of animals tested at different times after sensitization. The lymphoid-cell response indicated intenser reactions in the "11-day" group than among

the other animals. This surprising finding could not be confirmed by naked-eye assessment or general microscopy, but a similar trend was seen. It is known from earlier investigations (28) and from clinical experience (see 62) that in certain cases contact allergy gradually diminishes and finally disappears, but it has not previously been observed that the hypersensitivity may show a maximum at a particular time shortly after sensitization. Irrespective of the reason for this, the difference between the groups has proved convincingly demonstrable only by the new method.

Investigations were also carried out to assess the lymphoid-cell response in unsensitized guinea-pigs after application of sub-toxic doses of DNCB giving reactions not visible to the naked eye. Some increase was obtained, and this was rather greater with the larger of the doses. The solvent used, acetone, provoked no lymphoid-cell response.

On application of DNCB to the skin in a toxic dose (100 γ/sq. cm.) the lymphoid-cell response varied quite considerably among different animals, but was as a rule appreciably less than in the allergic reactions that showed the same intensity on naked-eye inspection. General microscopy usually disclosed a totally different picture, and seemed to be superior to the other methods for differentiating between allergic and toxic reactions. On the other hand, the lymphoid-cell response is probably a good measure of the allergic component in mixed reactions.

When croton oil was used to provoke a toxic reaction in DNCB-sensitized animals the lymphoid-cell response was slight, even when the naked-eye reaction was marked. The general microscopical picture here sometimes indicated a weak allergic reaction.

Thus the lymphoid-cell response occurring in toxic skin reactions differs from that seen in allergic reactions mainly in degree.

The findings of the investigation suggest that the lymphoid-cell response is a useful measure of allergic contact dermatitis, but it is not suitable for grading toxic reactions. In conjunction with general microscopy the method seems to be of value for differentiating between allergic and toxic reactions, especially when they are mixed.

When in guinea-pig the rubor and tumor due to vascular reaction as assessed by the naked eye are compared with the lymphoid-cell response in the epithelium, it is essential to bear in mind the two entirely different morphological criteria upon which the methods are based. It is not known which morphological changes are direct sequelae of the antigen-antibody reaction, and it is therefore difficult to say which is the most appropriate method of assessing the intensity of the allergic reaction. The vascular reaction would appear to be less specific, since both toxic and urticarial reactions have as a rule the same naked-eye appearance. The behaviour of the lymphoid cells during the early stages examined here would appear more characteristic of the allergic contact reactions.

Paper II

Since in delayed allergy it has proved impossible to demonstrate humoral antibodies responsible for the test reaction, the existence of "cell-bound" or cellular antibodies has been suggested.

In this investigation it is postulated that on sensitization to a simple chemical substance (contact allergy) a specific immune response is induced in certain lymphoid cells, irrespective of which theory of cell sensitization is accepted (7, 37, 43, 47, 55, see also 54). By division and differentiation these lymphoid cells form a "clon" of sensitized cells that, like other lymphocytes, can reach via the blood stream the reaction area in the skin.

It is not yet known, however, whether the accumulation of lymphoid cells in a test lesion in allergic contact dermatitis takes place at random or selectively from the blood. It is not unlikely that selection may be conditioned by immunological factors, resulting in accumulation of sensitized cells from a particular clon.

In order to be able to trace a sensitized cell from the moment it is formed until its possible infiltration into a test reaction some mode of labelling is required that either persists in the cell or is passed on to the progeny of that cell. Labelling of the nuclei is obtained with tritium thymidine (41), which is specifically incorporated into deoxyribonucleic acid (DNA) in proliferating cells preparing for mitosis. The daughter cells are therefore also labelled, but with an intensity that diminishes with increasing number of generations (5, 12, 21, 59, 60). The detection of H^3-thymidine-labelled cells is possible by microautoradiography (4, 36).

In this investigation guinea-pig cells were labelled by intramuscular injection of tritium thymidine (spec. act. 1.9 C per *m*mol, Schwartz Lab.), and 2,4-dinitrochlorobenzene (DNCB) was used for sensitization and testing. Sections of skin and lymph nodes and blood smears were examined autoradiographically by means of the stripping-film technique (56).

Three groups of animals were sensitized and injected with thymidine, as shown in Fig. 1.

In group D, in which the animals were sensitized before labelling, there ought, according to the above postulate, to be a clon of proliferating sensitized cells that are subsequently going to incorporate H^3-thymidine and thus become labelled. In group C labelling and sensitization were carried out at the same time. Investigations have shown that there is a latent period between the application of the sensitizing substance and the subsequent general cutaneous sensitization (30). This might mean that there is also a latent period before the formation and proliferation of sensitized cells. If this were the case few or no sensitized cells would become labelled from the start in these

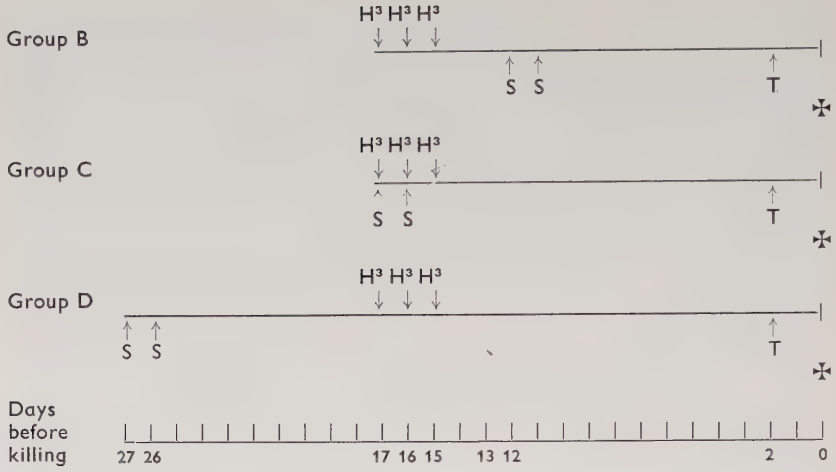

Fig. 1. S = sensitization. H^3 = H^3-thymidine-injection. T = epicutaneous test.

animals. If, on the other hand, proliferation of sensitized cells were to start immediately after the first contact with the antigen, a relatively large number of cells ought to be labelled at the time of testing and autoradiography. The animals of group B received their injections of H^3-thymidine before sensitization; at the time of labelling, therefore, unsensitized cells only can have been labelled. As can be seen from the figure, testing was carried out after the same interval following injection of the isotope in all three groups of animals.

Labelled and unlabelled lymphoid cells were counted in the epidermis within the test areas. The proportions of labelled lymphoid cells infiltrating the epidermis in the lesion were in group B 4.4 ($S.E. = 0.46$), group C 5.0 ($S.E. = 0.67$), and group D 7.8 ($S.E. = 0.95$), i.e. highest among animals sensitized before labelling. Group D differs from the other two, both individually and combined: D—B + C, $P < 0.01$; D—C, $P < 0.05$; D—B, $P < 0.01$.

Investigations were carried out to elucidate whether the difference between the groups can be explained by a more efficient labelling of all cells or of the lymphocyte population in the group-D animals. Judged after the ratio labelled/unlabelled epithelial cells in normal epidermis, there was a greater number of labelled epithelial cells in group B than in groups C and D. The percentage of labelled lymphoid cells in normal skin did not differ demonstrably in the three groups, but here again the figures tended to be higher among the animals of group B. Lymph nodes from intact hindquarters (i.e., the animals had not been sensitized, and no test had been done on the region drained)

from animals of group B and D were investigated, but no difference in the proportion of labelled lymphoid cells could be demonstrated between the two groups. To sum up, there was thus no evidence that more intense labelling of cells in group D would explain the result.

It is then conceivable that labelled lymphoid cells accumulated selectively at the test areas to a greater extent in group D than among other animals. In contrast to those of the other two groups, the group-D animals were sensitized before labelling, and the sensitization may have influenced the selection. One explanation would then be that the group-D animals had already developed a clon of sensitized cells many of which became labelled at the time of injection of tritium thymidine. The number of sensitized cells that were also labelled ought then to have been greater in group D than in the other two groups. These labelled and sensitized lymphoid cells then accumulated selectively at the test area. The greater number of labelled lymphoid cells in the tests of this group may therefore be evidence in favour of the existence of a clon of sensitized cells.

In groups B and C the test lesions contained labelled lymphoid cells, however. These labelled cells may have been unsensitized with regard to DNCB, but it is also possible that some initially labelled cells may subsequently have become sensitized during the simultaneous or later sensitization.

The findings in corium suggest that groups B and D did not differ with regard to the selection of infiltrating lymphoid cells. It is possible, however, that an unselected lymphoid-cell population had infiltrated the corium, and that only the cells that had accumulated in epidermis were selected.

The incidence of labelled mononuclear cells in the blood of the animals examined was only about 0.6%, whereas in epidermis the incidence of labelled lymphoid cells was about 5% both in normal skin and in tests among animals similarly treated. Even though it is difficult to make direct comparison between the degree of labelling in sections and smears, the finding would suggest that relatively young lymphoid cells were selectively finding their way to the epidermis. This is fully compatible with the fact that when labelling with tritium thymidine was done immediately before testing, a high proportion of the lymphoid cells infiltrating the test lesion became labelled and therefore represented a relatively young cell population (see also 44, 50).

The results I have obtained in actively-sensitized guinea-pigs provide evidence that selection takes place when lymphoid cells accumulate in the epidermis of tested skin. This selection appears to be influenced by sensitization, and one explanation would be that the selected cells belong to a cell clon induced by the sensitization.

Paper III:1

The fact that it is possible passively to transfer reactivity of the delayed type has been held as one of the most important features in evidence of an allergic genesis of such reactivity (13). Passive transfer has also been widely used in experiments, chiefly on animals (guinea-pigs) but also on man, designed to elucidate the type of cell that can be used, the number of cells necessary, the viability of the cells, and possibilities of using cell fractions (for ref. see 62). Different transfusion techniques have been studied, and experiments have also been carried out to trace the transfused cells in the recipient (see paper III:2). Microscopical examination of contact-allergic lesions provoked by epicutaneous application of allergen in passively-sensitized animals has been reported in only a few investigations. De Weck and Brun (68) were unable to detect any eczematous changes of the type seen in directly-sensitized animals in either ordinary skin or on the nipples with their thicker epidermis. This observation led Grimmer and Spier (33) to reproduce passive transfer of hypersensitivity to DNCB in guinea-pigs, and they found histological evidence of eczematous changes in test lesions of the skin.

In order further to elucidate this question, especially with regard to the infiltrating lymphoid cells, I examined the test lesions of recipients by three methods, (1) naked-eye inspection, (2) general microscopy, and (3) lymphoid-cell response in epidermis. Sensitization of the donors was carried out by the epicutaneous route, with the object of minimizing the formation of humoral antibodies (see 10, 62). The contact allergen used was 2,4-dinitrochlorobenzene, and to the test areas 9 or 20 γ DNCB per sq. cm. of skin was applied. Cell suspensions for transfusion were prepared from lymph nodes and thymus combined or from spleens. The cells introduced into each recipient were counted, and the viability of the cells was tested.

Assessed by naked-eye inspection several tests were positive, especially with the higher dose of antigen (20 γ DNCB/sq. cm. skin); general microscopy disclosed very slight or no cellular infiltration in the corium. In epidermis such morphological changes as are regularly present in the test lesions of directly-sensitized guinea-pigs were seen only within a few small areas. The lymphoid-cell response in test lesions examined 48 hours after transfusion and application of the test substance was slight. Reactions assesed as + by the naked eye differed significantly from the lymphoid-cell response in + reactions in directly-sensitized animals. Test lesions were also examined after 12 and 24 hours (the times were chosen with regard to the subsequent isotope investigation of labelled cells present in test lesions in the passively-sensitized animals). General microscopy showed the same picture at

all three times, whereas the lymphoid-cell responses in certain tests were slightly greater at 12 and 24 hours.

The results found by general microscopy and with the lymphoid-cell response seem to agree most nearly with the findings of de Weck and Brun that the same morphological changes in allergic test reactions in directly-sensitized guinea-pigs do not occur in passively-sensitized recipients. The characteristic eczematous changes described by Grimmer and Spier (basal spongiosis in the epidermis and lymphoid-cell infiltration) were observed, though only sporadically, and they were slight and limited in extent.

Summing up the results of all methods of assessment used here, it is found that the skin reaction of guinea-pigs passively sensitized to the contact allergen DNCB is localized largely to the corium. The vascular component predominates, and is most readily studied by naked-eye inspection paying regard to the degrees of redness and oedema. The cellular infiltration in both corium and epidermis would seem to be subordinate, at any rate from the quantitative point of view. It must be questioned whether the genesis of the test lesions is the same in directly- and indirectly-sensitized animals.

Paper III:2

The fate of cells transfused in passive transfer of delayed hypersensitivity to contact allergy has been studied by labelling them with fluorescent dyes (34) or isotopes (24, 35, 50, 52, 53). Fichtelius (24) transferred thymus lymphocytes labelled with radioactive phosphorus, and demonstrated that lymphocytes from DNCB-sensitized animals differed from those of unsensitized ones: he was unable to find conclusive evidence that the transferred cells reached the skin, which is in agreement with Turk's later findings (63).

In the present investigation contact test lesions and intact skin of recipients were subjected to microautoradiographic examination to elucidate, (1) the presence and localization of labelled lymphoid donor cells and (2) the occurrence of labelling of epithelial cells in the epidermis, indicating that reutilization of break-down products of DNA of donor cells had taken place.

Labelling of cells to be transfused was carried out with tritiated thymidine either *in vitro* in the prepared suspensions or *in vivo* by injection into the donors.

Cell suspensions labelled *in vitro* contained about 5% labelled cells, of which the great majority were heavily labelled. They also contained a certain amount of free H^3-thymidine which after transfusion gave rise to weak labelling of cells in the recipients. When reading the autoradiograms, therefore, only heavily-labelled cells were considered. No

cells that conceivably could have originated in donor animals were found either in the epidermis or corium of normal or tested skin 24 or 48 hours after transfusion and application of the allergen.

In addition to these animals, a series of recipients were passively sensitized with cells from sensitized donors into which H^3-thymidine had been injected *in vivo*. The cell suspensions contained about 15% of labelled cells. Skin samples were taken from normal skin and test areas treated with 9 and 20γ of DNCB/sq. cm. 12, 24, and 48 hours after transfusion and application of the allergen .

Again, no autoradiographic evidence was forthcoming to suggest that the transfused lymphoid cells necessarily accumulate at the site of epicutaneously-provoked, naked-eye-positive reactions. No definite conclusions concerning the presence of donor cells can be drawn, however, because only a limited number of lymphoid cells transfused were labelled with isotope.

These results are in accordance with those recently reported by McCluskey *et al.* (50) and Hamilton and Chase (35), but not with those of Najarian and Feldman (52, 53). All of these workers used H^3-thymidine for labelling the cells, but differences in methods between the investigations make comparison uncertain. This study differs from the others in that the sensitization of the donor was done epicutaneously, and the cell suspensions were transfused intraperitoneally to the recipients.

Recent research has indicated that deoxyribonucleic acid (DNA) is reutilized *in vivo* in epidermal cells (15, 25). Examination of my autoradiograms of animals transfused with lymphoid cells labelled *in vivo* disclosed grains over the nuclei of occasional epithelial cells which apparently were caused by true isotope labelling. This might suggest that reutilization of labelled products of DNA breakdown originating from donor cells had taken place.

If reutilization of labelled DNA products by the epithelial cells of passively-sensitized recipients does indeed take place, it ought to do so only to a very limited extent. The autoradiographic identification of weak labelling in stripping films is rendered difficult by background labelling or fogging, which cannot as a rule be distinguished from isotope-induced grains by ordinary microscopy. In an attempt to detect even minor quantities of isotope, skin sections from recipients and isotope-free control sections were mounted on the same slide. Detailed counting failed to reveal any difference between recipients and controls.

Consequently, on the basis of these results it has proved impossible to confirm the presence of reutilized labelled DNA material in the epithelial cells.

Acknowledgements

These investigations were carried out at the Department of Histology, University of Uppsala. I am greatly indebted to the former head of the department, Professor M. Wrete, and his successor, Professor K.-E. Fichtelius, for generously putting at my disposal the resources of the department.

I wish to thank Professor N. Thyresson, head of the Department of Dermatology, for his kind encouragement and constant support in my work, and Professor Fichtelius, who introduced me to the lymphoid cells and infected me with interest for their role in allergy, for his stimulating enthusiasm and never-failing help and guidance throughout these investigations. Docent H. Diderholm taught me the autoradiographic technique and gave me much good advice. I also extend my thanks to my colleagues and other staff members of the Departments of Dermatology and Histology, who have helped me in many ways. Mr. Adam Taube, Fil. lic., lecturer in statistics, advised me concerning the statistical aspects of the work. Dr. Marcia Skogh helped me with preparation and translation of the manuscript. I gladly acknowledge the excellent technical assistance of Mrs. Maj-Lis Johansson, Mrs. Ingrid Skeppstedt, and Mrs. Ingegerd Spove.

The investigations were supported by grants from the Faculty of Medicine, University of Uppsala, Jörgen Schaumann's Fund and the Swedish Medical Research Council.

References

1. Baer, R. L., Rosenthal, St. A. and Sims, C.: The allergic eczemalike reaction and the primary irritant reaction. A histologic comparison of their evolution in the acanthotic skin of guinea pigs. *Arch. Derm. (Chicago) 76,* 549, 1957.
2. Bandmann, Hans-Jürgen: Beitrag zur Histopathologie allergischer epicutaner Testreaktionen. *Der Hautarzt 11,* p. 258, 310, 355, 393. 1960.
3. Bloch, B. and Steiner-Wourlisch, A.: Die Sensibilisierung des Meerschweinchens gegen Primelu. *Arch. Derm. Syph. (Berlin) 162,* 349, 1930.
4. Boyd, G. A.: *Autoradiography in Biology and Medicine.* Acad. Press Inc., New York, 1955.
5. Braunsteiner, M., Höfer, R. and Sailer, S.: Der Lymphocyt. *Deutsch Med. Wschr. 15,* 721, 1961.
6. Burdick, K. H.: The influence of whole body X-irradiation of epidermal hypersensitivity: Correlation with lympocyte response. *Acta Dermato-vener. 37,* 110, 1957.
7. Burnet, F. M.: *The Clonal Selection Theory of Acquired Immunity.* University Press, Cambridge, 1959.
8. Chase, M. W.: The cellular transfer of cutaneous hypersensitivity. *J. Bact. 51,* 643, 1946.
9. Chase, M. W.: Experimental sensitization with particular reference to picryl chloride. *Int. Arch. Allerg. 5,* 162, 1954.
10. Chase, M. W.: Models for hypersensitivity studies. *Cellular and Humoral Aspects of the Hypersensitivity States.* Ed. Lawrence, Hoeber, New York, 1959.

11. Chase, M. V.: Chemical considerations of drug hypersensitivity. *Allergology—Pro IVth Intn. Cong. Allerg.* Ed. Brown. 92, 1961.
12. Cronkite, E. P., Bond, V. P., Fliedner, T. M. and Killman, S. A.: The use of tritiated thymidine in the study of haemopoietic cell proliferation. *Haemopoesis.* J. & A. Churchill, Ltd., London, 70, 1960.
13. Crowle, A. J.: *Delayed Hypersensitivity in Health and Disease,* Springfield, Ill. Charles C. Thomas, 1962.
14. Cohen, S. G., Mayer, L. D. and Criep, Z. H.: The effect of X-irradiation on experimentally produced cutaneous sensitivity. *J. Invest. Derm. 16,* 91, 1951.
15. Diderholm, H., Fichtelius, K. E. and Linder, O.: Availability time of H^3-thymidine *in vivo. Exp. Cell Res. 27,* 431, 1962.
16. Eisen, H. N.: Hypersensitivity to simple chemicals. *Cellular and Humoral Aspects of the Hypersensitive States.* Ed. Lawrence, 89, 1959.
17. Eisen, H. N., Orris, L. and Belman, S.: Elicitation of delayed allergic skin reactions with haptens: the dependence of elicitation on hapten combination with protein. *J. Exp. Med. 95,* 473, 1952.
18. Epstein, W. L.: Induction of allergic contact dermatitis in patients with the lymphoma leukemia complex. *J. Invest. Derm. 30,* 39, 1958.
19. Epstein, W. L., and Myock, R. L.: Induction of allergic contact dermatitis with sarcoidosis. *Proc. Soc. Exp. Biol. Med. 96,* 786, 1957.
20. Epstein, W. L. and Kligman, A. M.: Some factors affecting the reaction of allergic contact dermatitis. *J. Invest. Derm. 33,* 231, 1959.
21. Everett, N. B., Reinhardt, W. O. and Yoffey, J. M.: The appearance of labeled cells in the thoracic duct lymph of the guinea pig after the administration of tritiated thymidine. *Blood 15,* 82, 1960.
22. Fagraeus, A.: Antibody production in relation to the development of plasma cells. *Acta Med. Scand. Supp. 204,* 1948.
23. Fazio, M., Calciati, A. and Depaoli, M.: Dermatitie allergica da contatto nel linfogranuloma maligno. *Minerva Derm. 37,* no. 8, 249, 1962.
24. Fichtelius, K. E.: The influence on lymphocytes of sensitization to dinitrochlorobenzene shown by transfusion of labelled lymphocytes. *Acta Dermatovener. 39,* 75, 1959.
25. Fichtelius, K. E. and Diderholm, H.: Autoradiographic analysis of the accumulation of lymphocytes in wounds. *Acta Path. Microbiol. Scand. 52,* 11, 1961.
26. Fischer, J. P. and Cooke, R. A.: Experimental toxic and allergic contact dermatitis. II. A histopathologic study. *J. Allerg. 29,* 411, 1958.
27. Frei, W.: Über willkürliche Sensibilisierung gegen chemisch-definierte Substanzen. *Klin. Wschr. 1,* 1026, 1928.
28. Frey, J. R.: Quantitative Untersuchungen bei epidermaler Sensibilisierung von Meerschweinchen mit Dinitrochlorbenzol. *Dermatologica 102,* 1, 1951.
29. Frey, J. R. and Wenk, P.: Experimentelle Untersuchungen zur Pathogenese des Kontaktekzems. *Dermatologica 112,* 264, 1956.
30. Frey, J. R. and Wenk, P.: Über die Funktion der regionalen Lymphknoten bei der Entstehung des Dinitrochlorbenzol-Kontaktekzems am Meerschweinchen. *Dermatologica 116,* 243, 1958.
31. Gell, P. G. H.: Cellular hypersensitivity. *Int. Arch. Allerg. 18,* 39, 1961.
32. Good, R. A., Zak, S. J., Jensen, D. R., Pappenheimer, A. M. Jr.: Delayed allergy and agamma-globulinemia. *J. Clin. Invest. 36,* 894, 1957.
33. Grimmer, H. and Spier, H. W.: Histologische Verifizierung der passiven Übertragbarkeit des tierexperimentellen Kontaktekzems. *Hautarzt 12,* 300, 1961.
34. Hagerman, G.: How is epidermal hypersensitivity transmitted through lymphocytes? *Acta Dermato-vener. 34,* 51, 1954.
35. Hamilton, L. H. and Chase, M. W.: Labelled cells in the cellular transfer of delayed hypersensitivity. *Fed. Proc. 21,* 40, 1962.
36. Harbers, E.: Autoradiographie als histochemisches Untersuchungsverfahren. *Handbuch der Histochemie,* 1, 400, 1958. Gustav Fischer Verlag, Stuttgart.

37. Haurowitz, F.: Theories of Antibody Formation. *The Nature & Significance of the Antibody Response*. Ed. Pappenheimer, Columbia Univ. Press. New York, 1953.
38. Haxthausen, H.: The pathogenesis of allergic eczema elucidated by transplantation experiments on identical twins. *Acta Dermato-vener. 23,* 438, 1943.
39. Haxthausen, H.: Studies on the role of the lymphocytes as transmitter of hypersensitiveness in allergic eczema. *Acta Dermato-vener. 27,* 275, 1947.
40. Haxthausen, H.: Passive transmission of dinitrochlorobenzene allergy with white blood cells from sensitized guinea pigs. *Acta Dermato-vener. 31,* 659, 1951.
41. Hughes, W. L., Bond, V. P., Brecher, G., Cronkite, E. P., Painter, R. B., Quastler, H. and Sherman, F. G.: Cellular proliferation in the mouse as revealed by autoradiography with tritiated thymidine. *Proc. Nat. Acad. Sci. USA 44,* 476, 1958.
42. Inderbitzin, Th.: The relationship of lymphocytes, delayed cutaneous allergic reactions and histamine. *Int. Arch. Allerg. 8,* 150, 1956.
43. Jerne, N. K.: The natural-selection theory of antibody formation. *Proc. Nat. Acad. Sci. USA 41,* 849, 1955.
44. Kosunen, T. U., Waksman, B. H., Flax, M. H. and Tihen, W. S.: Radioautographic study of cellular mechanisms in delayed hypersensitivity. Delayed reactions to tuberculin and purified protein in rat and guinea-pig. *Immunology 6,* 276, 1963.
45. Landsteiner, K. and Chase, M. W.: Experiments on transfer of cutaneous sensitivity to simple compounds. *Proc. Soc. Exp. Biol. Med. 49,* 688, 1942.
46. Landsteiner, K. and Jacobs, J.: Studies on the sensitization of animals with simple chemical compounds. *J. Exp. Med. 61,* 643, 1936.
47. Lederberg, J.: Genes and antibodies. *Science 129,* 1649, 1959.
48. Maguire, Henry C. Jr. and Maibach, Howard, I.: Effect of X-Ray lymphopenia on contact dermatitis. *Arch. Derm. 88,* 8, 136, 1963.
49. Mayer, R. L.: The significance of cross-links in the formation of hapten-carrier complexes. *Progr. Allerg. 4,* 79, 1956.
50. McCluskey, R. T., Benacerraf, B. and McCluskey, J. W.: Studies in the specificity of the cellular infiltrate in delayed hypersensitivity reactions. *J. Immun. 90,* 466, 1963.
51. Miescher, G.: *Handbuch der Haut- und Geschlechtskrankheiten.* J. Jadassohn. Ergänzungswerk Band II, Teil I, p. 1. Springer-Verlag, 1962.
52. Najarian, J. S. and Feldman, J. D.: Passive transfer of contact sensitivity by tritiated thymidine-labelled lymphoid cells. *J. Exp. Med. 117,* 775, 1963.
53. Najarian, J. S. and Feldman, J. D.: Specificity of passively transferred delayed hypersensitivity. *J. Exp. Med. 118,* 341, 1963.
54. Nossal, C. J. V.: Cellular genetics of immune responses. *Advances in Immunology,* Academic Press, New York and London, vol. 2, 163, 1962.
55. Pauling, L.: A theory of the structure and process of formation of antibodies. *J. Amer. Chem. Soc. 62,* 2640, 1940.
56. Pelc, S. R.: The stripping-film technique of autoradiography. *Int. J. Appl. Radiat. 1,* 172, 1956.
57. Raffel, Sidney: The Immune Response in Hypersensitivity. *Allergology — Pro. IV Intn. Cong. Allergology.* Ed. Brown, 139, 1961.
58. Rostenberg, A. Jr., McCraney, H. C., Bluefarb, S. M.: Immunologic studies in the lymphoblastomas. *J. Invest. Derm. 26,* 209, 1956.
59. Schooley, J. C., Bryant, B. J. & Kelly, L. S.: Preliminary autoradiographic observations of cellular proliferation in lymphoid tissues, using tritiated thymidine. *The Kinetics of Cellular Proliferation.* Ed. F. Stohlman, Grune & Stratton, New York, 208, 1959.
60. Schooley, J. C. and Berman, I.: Morphologic and autoradiographic observations of H^3-thymidine-labeled thoracic duct lymphocytes cultured *in vivo*. *Blood 16,* 1133, 1960.

61. Skog, E.: Experimental studies on hypersensitivity to 2,4-dinitrochlorobenzene. I. Passive transfer of hypersensitivity to 2,4-dinitrochlorobenzene. *Acta Dermato-vener. 36,* 93, 1955.
62. Storck, H.: *Handbuch der Haut- und der Geschlechtskrankheiten.* J. Jadossohn, Ergänzungswerk Band II, Teil I, p. 113, Springer-Verlag, 1962.
63. Turk, J. L.: The passive transfer of delayed hypersensitivity in guinea pigs by the transfusion of isotopically-labelled lymphoid cells. *Immunology 5,* 478, 1962.
64. Waksman, B. H.: The toxic effects of the antigen-antibody reaction on the cells of hypersensitive reactors. *Cellular and Humoral Aspects of the Hypersensitive States.* Ed. Lawrence, 123, 1959.
65. Waksman, B. H.: A comparative histopathological study of delayed hypersensitive reactions. *Ciba Found. Sympos. on Cellular Aspects of Immunity,* Churchill, London, 280, 1960.
66. Waksman, B. H.: Tissue damage in the "delayed" (cellular) type of hypersentitivity. *Mechanism of Cell and Tissue Damage Produced by Immune Reactions. II Intn. Symp. Immunopath. 1961.* Ed. Grabar & Miescher, 146, 1962.
67. Waksman, B. H. and Arbouys, S.: Use of specific "lymphocyte" antisera to inhibit hypersensitive reaction of the delayed type. *Sympos. on Mechanisms of Antibody Formation.* Czech. Acad. Sciences, Prague, p. 165, 1960.
68. de Weck, A. and Brun, R.: De l'éczema expérimental. *Acta Dermato-vener. 36,* 360, 1956.
69. Wilhelm, R. E., Fisher, J. P. and Cooke, R. A.: Experimental depletion of mononuclear cells for the purpose of investigating reactions of the allergic contact type. *J. Allerg. 29,* 493, 1958.
70. Wesslén, T.: Passive transfer of tuberculin hypersensitivity by viable lymphocytes from the thoracic duct. *Acta Tuberc. Scand. 26,* 38, 1952.

ACTA UNIVERSITATIS UPSALIENSIS

Abstracts of Uppsala Dissertations in Medicine

Editor: The Dean of the Faculty of Medicine

---

1. TORKEL SCHOLANDER: Studies in the Habituation of Autonomic Response Elements. 1961. Price Sw. Cr. 2:—
2. WOLFGANG J. KIRSTEN: Studies in Quantitative Organic Microanalysis. 1961. Price Sw. Cr. 2:—
3. JOHN STRANDH: Chemical and Biophysical Studies of Microscopic Structures in Compact Bone. 1961. Price Sw. Cr. 2:—
4. BRITA LITHANDER: Kliniska och experimentella undersökningar med ekoencefalografi. (Clinical and Experimental Studies in Echo-encephalograpy.) 1961. Price Sw. Cr. 2:—
5. ANDERS PARROW: Studies on the Triiodothyronine Uptake by Erythrocytes. 1962. Price Sw. Cr. 2:—
6. LORENTZ ENGSTRÖM: Studies on Alkaline Phosphatase. Incorporation of Phosphate from Inorganic Phosphate and Glucose-6-Phosphate, and Mechanism of Action of the Enzyme. 1962. Price Sw. Cr. 2:—
7. ANDERS BILL: Studies on Uveal Circulation and Aqueous Humour Drainage. 1962. Price Sw. Cr. 4:—
8. CLAES F. HÖGMAN: Interaction between Human Blood Group Antibodies and Fetal Cells in Tissue Culture. 1962. Price Sw. Cr. 4:—
9. HANS R. ULFENDAHL: Studies on the Intrarenal Separation of Red Cells and Plasma. 1962. Price Sw. Cr. 4:—
10. NILS-HOLGER ARESKOG: Experimental Studies on Myocardial Function with special reference to ionic factors and the influence of hormones and cardiac glycosides in the dog heart-lung preparation. 1962. Price Sw. Cr. 4:—
11. KARL-ERIK FJELLSTRÖM: Studies on Separation of Complement Active Serum Proteins and Preparation of Complement Reagents. 1963. Price Sw. Cr. 6:—
12. CLAES HELLERSTRÖM: Studies on the Pancreatic Islets with regard to the two types of A cells, the influence of pregnancy on the islet organ and a technique for isolation of intact islets of mammals. 1963. Price Sw. Cr. 6:—
13. JOHAN KILLANDER: Separation of Immunoglobulins and other Plasma Proteins by Gel Filtration, Zone Electrophoresis and Ion Exchange Chromatography. 1964. Price Sw. Cr. 6:—
14. OVE GROTH: Lymphoid Cells in Allergic Contact Dermatitis in Guinea-Pigs. 1964. Price Sw. Cr. 6:—

Doctoral dissertations in Medicine are published either as monographs or as summaries of articles previously printed. It is these summaries that are collected in the series "Abstracts of Uppsala Dissertations in Medicine".

Distributed by

Almqvist & Wiksell

Gamla Brogatan 26

STOCKHOLM, SWEDEN

Price Sw. Cr. 6:—

ALMQVIST & WIKSELL 642119